K120946

510(k) Summary
ALPHA Cryptococcal Antigen EIA

DEC 17 2012

This 510(k) summary is submitted in accordance with 21 CFR §807.92

Owner: Immuno-Mycologics, Inc.
2700 Technology Place
Norman, OK 73071
Tel: 405-360-4669
Fax: 405-364-1058
Contact: Dr. Sean K. Bauman, President & CEO

Prepared: February 27th, 2012

Trade Name: ALPHA Cryptococcal Antigen EIA

Common Name: Cryptococcus Antigen EIA

Classification Name: Antigen, Elisa, Cryptococcus

Regulation: 866.3165

Predicate Device: Meridian's Premier™ Cryptococcal Antigen EIA, K904393

Intended Use: The ALPHA Cryptococcal Antigen enzyme immunoassay (CrAg EIA) is a qualitative or semi-quantitative (titration) test system for the detection of capsular polysaccharide antigens of Cryptococcus species complex (Cryptococcus neoformans and Cryptococcus gattii) in serum and cerebrospinal fluid (CSF). The ALPHA Cryptococcal Antigen Enzyme Immunoassay is an assay which can be used as an aid in the diagnosis of cryptococcosis. Test results are to be used in conjunction with information available from the patient clinical evaluation and other diagnostic procedures.

Device Description:

The ALPHA Cryptococcal Antigen Enzyme Immunoassay (EIA) is a direct immunoenzymatic sandwich microplate assay which detects Cryptococcus antigens in serum and CSF. Anti-Cryptococcus antibodies bound to microwell plates are used as capture antibodies, and horseradish peroxidase (HRP)-conjugated anti-Cryptococcus antibodies are used as detect antibodies. The positive control and standard curve material are composed of cryptococcal capsular polysaccharide antigen in a buffered protein solution with a preservative.
In the qualitative procedure, specimens are analyzed undiluted. In the titration procedure, specimens are analyzed after serial dilution in specimen diluent. Either serum or CSF is added to the microwells coated with the capture antibodies and incubated. If the patient specimen contains cryptococcal antigens that are recognized by the capture antibodies, those antigens will

become bound to the microwells. The microwells are washed to remove unbound patient material, and HRP-conjugated detect antibody is added to the wells. If Cryptococcus antigens are bound to the microwells by the capture antibodies, the detect antibody will also become bound to the microwells. The wells are then washed to remove any unbound detect antibody. Next, tetramethylbenzidine (TMB) substrate is added to the microwells, and in the presence of HRP, a blue color will develop. The reaction is stopped by the addition of a stop solution. The optical density (OD) is determined with a microplate reader at 450 nm with reference at 630 nm (reference is optional).

Comparison with Predicate:
A comparison of the similarities and differences between the ALPHA Cryptococcal Antigen EIA and the predicate device is presented in the tables below (Table 1).

Table 1. Similarities and Differences between ALPHA Cryptococcal Antigen EIA and Premier Cryptococcal Antigen EIA

SIMILARITIES		
Assay Feature	**ALPHA Cryptococcal Antigen EIA New Device**	**Premier Cryptococcal Antigen EIA K904393**
Intended Use		
Intended Use	Detection of capsular polysaccharide antigens	Detection of capsular polysaccharide antigens
Indication For Use	Aid in the diagnosis of cryptococcosis	Aid in the diagnosis of cryptococcosis
Device Description		
Assay Principle	EIA	EIA
Sample Matrix	Serum & CSF	Serum & CSF
Assay components	Antibody coated 96-well microplate, wash buffer, positive control, enzyme conjugate, TMB substrate, stop solution, sample diluent	Antibody coated 96-well microplate, wash buffer, positive control, enzyme conjugate, TMB substrate, stop solution, sample diluent
Detection Chemistry	HRP + TMB	HRP + TMB
Specimen pre-treatment?	none	none
Controls/Standard	Cryptococcal Antigen	Cryptococcal Antigen
Microplate	96-well microplate coated with antigen	96-well microplate coated with antigen

DIFFERENCES		
Assay Feature	**ALPHA Cryptococcal Antigen EIA New Device**	**Premier Cryptococcal Antigen EIA K904393**
Device Description		
Output	Positive or Negative Only	Positive, Negative, and Indeterminate
Reagent Application	Pipette or equivalent	Reagent Droppers
Optional Visual Read	No	Yes

Analytical Performance Summary

A. Precision Studies

Precision Test Methods:

The ALPHA Cryptococcal Antigen EIA was evaluated for reproducibility and precision by spiking serum and mock CSF with cryptococcal antigen to produce a panel consisting of a negative sample, a high negative (C_5) sample, a low positive sample and a moderate positive sample. This panel was tested twice per day at three sites [Immuno-Mycologics, Inc., a national reference lab, and a clinical lab] with a total of five operators over a five-day period in order to determine both the inter-lab and the intra-lab reproducibility and precision of the assay. The results of this study are shown in the table below.

		IMMY			National Reference Lab			Clinical Lab			Combined Data (3 Sites)		
Description	Type	Ave O.D.	Std Dev	%CV	Ave O.D.	Std Dev	%CV	Ave O.D.	Std Dev	%CV	Ave O.D.	Std. Dev.	%CV
Blank	Control	0.066	0.012	17.8	0.000	0.0027	3686.2	0.075	0.005	7.0	0.046	0.034	74.67
CRYPC1	Control	1.814	0.078	4.3	1.973	0.1083	5.5	2.473	0.158	6.4	2.059	0.295	14.34
Negative	Serum	0.022	0.011	48.7	0.012	0.0058	48.8	0.019	0.005	25.4	0.018	0.009	50.25
High Negative	Serum	0.040	0.008	19.2	0.028	0.0077	27.7	0.035	0.007	20.0	0.034	0.009	26.51
Low Positive	Serum	0.420	0.041	9.7	0.338	0.0436	12.9	0.372	0.038	10.3	0.377	0.053	14.16
Moderate Positive	Serum	1.683	0.229	13.6	1.666	0.1281	7.7	1.959	0.205	10.5	1.756	0.229	13.05
Negative	CSF	0.065	0.018	27.8	0.058	0.0087	15.1	0.099	0.010	10.0	0.072	0.022	29.85
High Negative	CSF	0.179	0.020	11.1	0.174	0.0190	10.9	0.298	0.027	9.2	0.211	0.059	28.09
Low Positive	CSF	0.346	0.028	8.0	0.339	0.0319	9.4	0.533	0.039	7.3	0.397	0.092	23.27
Moderate Positive	CSF	0.629	0.039	6.2	0.606	0.0443	7.3	0.929	0.056	6.0	0.707	0.149	21.13

Below is the data from the reproducibility study presented by percent positive for each specimen type.

Serum	Site 1 % Positive	Site 2 % Positive	Site 3 % Positive	Overall % Positive
Negative	0%	0%	0%	0%
High Negative	0%	0%	0%	0%
Low Positive	100%	100%	100%	100%
Moderate Positive	100%	100%	100%	100%

CSF	Site 1 % Positive	Site 2 % Positive	Site 3 % Positive	Overall % Positive
Negative	0%	0%	0%	0%
High Negative	0%	0%	83%	24%
Low Positive	100%	100%	100%	100%
Moderate Positive	100%	100%	100%	100%

B. Analytical Sensitivity (Lower limits of the assay)

LoB and LoD Testing Methods

Analytical sensitivity was estimated at IMMY according to Clinical and Laboratory Standards Institute (CLSI) EP-17A. The limit of the blank (LoB) was estimated by running 80 replicates of normal human serum and 80 replicates of artificial CSF. The initial limit of detection (LoD) was estimated by running 20 replicates of 4 concentrations near the LoB in both serum and CSF.

The LoD was established by testing 24 replicates of both serum and CSF spiked with Cryptococcal antigen at a range of concentrations near the initial LoD. Results were considered positive if they yielded a blanked OD that was greater than the cut-off.

Analysis shows that for serum the LoD was 5.3 ng/ml. For CSF, the LoD was 1.7 ng/ml.

C. Analytical Specificity (Cross-Reactivity)

Analytical specificity for the ALPHA Cryptococcal Antigen EIA was determined by running potentially cross-reacting medical conditions unrelated to cryptococcosis. The following specimens were run on one lot of the ALPHA Cryptococcal Antigen EIA. A total of 118 serum specimen and 15 fungal culture filtrates were tested. Culture filtrates were tested at three different dilutions: undiluted, 1:10, and 1:100. Dilutes were made in 1X Specimen Diluent. Percent positive was determined for each condition.

As expected, some fungal cross-reactivity was observed. One *Aspergillus* galactomannan positive specimen (positive by Bio-Rad's Platelia™ *Aspergillus* EIA) was positive in the ALPHA Cryptococcal Antigen EIA and *Paracoccidioides brasiliensis* culture filtrates undiluted and 1:10 were positive as well. The total percent positive for fungal pathologies was 3.9%, well below the acceptance criteria of 10%. However, *Paracoccidioides brasiliensis* Culture Filtrate had a total percent positive of 67%.

Rheumatoid factor is known to cause false-positive results in the Cryptococcal latex agglutination method. Rheumatoid factor did not cause false positives in the ALPHA Cryptococcal Antigen EIA between the range of 112 IU/ml and 6479 IU/ml. The normal reference range for rheumatoid factor is 0-14 IU/ml.

D. Interference Studies

In addition to the cross-reactivity study, interference testing was also performed on five icteric, five hemolyzed, and five lipemic serum specimens. Each specimen was spiked with cryptococcal antigen at three times the C95 concentration. All specimens were then tested at IMMY, on one lot of the ALPHA Cryptococcal Antigen EIA in triplicate: spiked and unspiked.

All of the unspiked specimens had negative results on the ALPHA Cryptococcal Antigen EIA. All spiked specimens were positive, thus, these types of serum specimens do not interfere with the ALPHA Cryptococcal Antigen EIA.

E. High Dose Hook Effect

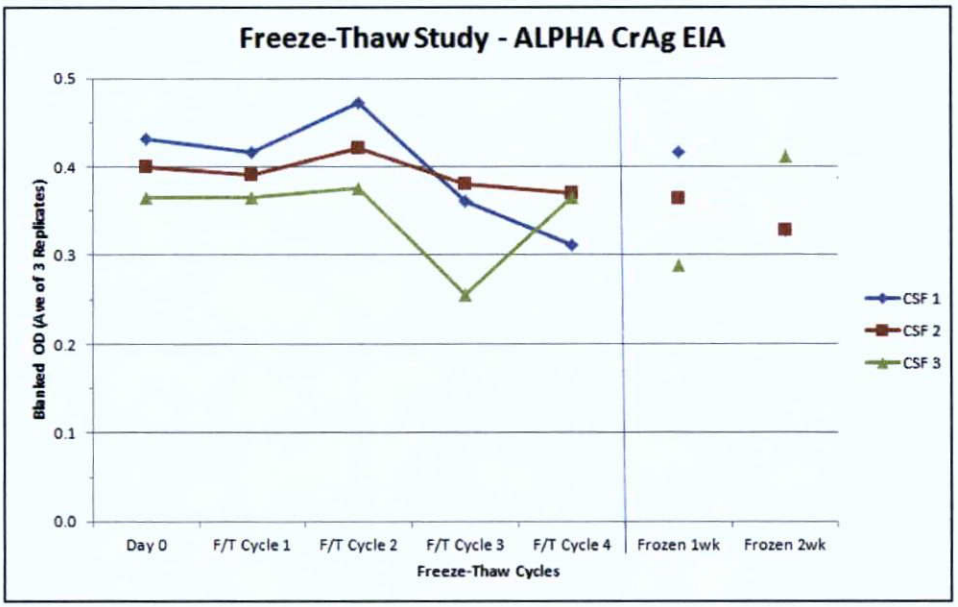
The effects of high doses were determined by spiking a serum specimen pool that was negative by the IMMY Latex-*Cryptococcus* Antigen Detection System and ALPHA Cryptococcal Antigen EIA, with cryptococcal antigen at 1 mg/ml and testing it in triplicate at IMMY on one lot of ALPHA Cryptococcal Antigen EIA, according to the package insert.

It was observed that at extremely high concentrations, the signal can be reduced due to the high dose hook effect. Although the signal is reduced leading to lower than expected EIA values, the result is still positive. The semi-quantitative titration procedure can differentiate between a low EIA score due to the high dose hook effect and a low EIA score due to low concentrations of Cryptococcal Antigen.

F. Specimen Acceptance Criteria – Freeze-Thaw Study

Three CrAg-Negative human CSF specimens and four CrAg-Negative human serum specimens were spiked with concentrations of Cryptococcal Antigen near and slightly above the cutoff of the assay. The specimens were tested on the ALPHA CrAg EIA immediately after spiking and then they were frozen at < -20 C for the Day 0 time point. The specimens were then divided into three aliquots and tested as follows:

- Aliquot 1 – Tested daily in triplicate, subjected to a freeze-thaw cycle each day
- Aliquot 2 – Frozen for 1 week, thawed and tested in triplicate
- Aliquot 3 – Frozen for 2 weeks, thawed and tested in triplicate



Specimens may be stored for up to one week at ≤-20 °C. Reductions in OD values after one week of specimen storage at ≤ -20 °C and after multiple freeze thaws may occur. Since a reduction in OD values was observed, it is possible that a fresh, very low-positive specimen (near 0.300 Blanked OD) could become negative if it is stored for one week. If possible, multiple freeze-thaw cycles should be avoided to minimize any effects due to specimen storage.

G. Linearity

N/A

H. Assay cut-off

The assay cutoff was determined through ROC analysis using the IMMY CrAg Lateral Flow Assay (LFA) as the means of classifying a specimen as a true positive or negative. The

intent of this classification system was to match the sensitivity and specificity of this assay to the sensitivity and specificity of the LFA.

ROC Analysis was performed on a dataset consisting of 995 combined serum and CSF specimens.

ROC Analysis suggested a blanked OD cutoff value of greater than 0.265 which yielded a sensitivity of 97.4% and a specificity of 99.9% when compared to the IMMY CrAg LFA as the reference method.

This cutoff was confirmed through the method comparison comparing the results from the IMMY Cryptococcal Antigen EIA to the predicate device.

Cut-Off Conclusions:

In summary our cut-offs are defined based on the blanked OD from the ROC analysis:

$x \leq 0.265$	Negative
$x > 0.265$	Positive

F. Method Comparison

Comparison to Another Manufacturer's Device

A three-site (IMMY and two national reference laboratories) split-specimen comparison study was performed on both serum and CSF specimens that had been submitted for cryptococcal antigen EIA testing. A total of 1782 specimens (CSF n=426; serum n=1356) were tested in both the ALPHA Cryptococcal Antigen EIA and a commercial Cryptococcal antigen EIA according to their respective package inserts.
The resulting data from the split sample comparison is shown in the tables below.

Serum 2x2 Contingency Table

	Commercial EIA (+)	Commercial EIA (-)
IMMY EIA (+)	131	28
IMMY EIA (-)	2	1195

CSF 2x2 Contingency Table

	Commercial EIA (+)	Commercial EIA (-)
IMMY EIA (+)	29	3
IMMY EIA (-)	2	392

The combined results, excluding indeterminates, were analyzed for percent agreement positive, percent agreement negative and overall percent agreement according to Clinical and Laboratory Standards Institute (CLSI) EP12-A2. The results of this analysis are shown in the table below.

	Dataset	Point Estimate	95% Confidence Interval
% Agreement Positive	Serum Only	98.5%	94.7% - 99.6%

% Agreement Negative	Serum Only	97.7%	96.7% - 98.4%
% Agreement Positive	CSF Only	93.5%	79.3% - 98.2%
% Agreement Negative	CSF Only	99.2%	97.8% - 99.7%

Comparison to IMMY CrAg Lateral Flow Assay

A three-site (IMMY and two national reference laboratories) split-specimen comparison study was performed on both serum and CSF specimens that had been submitted for cryptococcal antigen EIA testing. A total of 1782 specimens (CSF n=426; serum n=1356) were tested in both the ALPHA Cryptococcal Antigen EIA and the IMMY Cryptococcal Antigen Lateral Flow Assay (LFA) according to their respective package inserts.

The ALPHA Cryptococcal Antigen EIA performs similarly to the IMMY Cryptococcal Antigen Lateral Flow Assay (LFA) with 96.9% and 93.8% Agreement Positive, and 99.8% and 99.5% Agreement Negative to the LFA in serum and CSF, respectively.

Conclusion

The information submitted in this premarket notification is complete and supports a substantial equivalence decision.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

Immuno-Mycologics, Inc.
c/o Sean K. Bauman, Ph.D.
2700 Technology Place
Norman, OK 73071

DEC 17 2012

Re: k120946
Trade/Device Name: ALPHA Cryptococcal Antigen EIA
Regulation Number: 21 CFR §866.3165
Regulation Name: Cryptococcus neoformans serological reagents
Regulatory Class: Class II
Product Code: MDU
Dated: October 25, 2012
Received: October 26, 2012

Dear Dr. Bauman:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set

forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of In Vitro Diagnostics and Radiological Health at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Uwe Scherf for

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
And Radiological Health
Center for Devices and Radiological Health

Enclosure

Indications for Use Statement

510(k) Number (if known): K120946

Device Name: ALPHA Cryptococcal Antigen Enzyme Immunoassay

Indications for Use: The ALPHA Cryptococcal Antigen enzyme immunoassay (CrAg EIA) is a qualitative or semi-quantitative (titration) test system for the detection of capsular polysaccharide antigens of Cryptococcus species complex (Cryptococcus neoformans and Cryptococcus gattii) in serum and cerebrospinal fluid (CSF). The ALPHA Cryptococcal Antigen Enzyme Immunoassay is an assay which can be used as an aid in the diagnosis of cryptococcosis. Test results are to be used in conjunction with information available from the patient clinical evaluation and other diagnostic procedures.

Prescription Use X AND/OR Over-The-Counter Use
(Part 21 CFR 801 Subpart D) (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) k 120946